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SYNTHESIS, CYTOTOXIC AND ANTIOXIDANT EVALUATION OF PYRIMIDINE DERIVATIVES DERIVED FROM NOVEL CHALCONES

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ABSTRACT

Cancer is a fatal disease with a high mortality rate, followed by infectious and cardiovascular disorders. Consequently, the pursuit of effective and safe anticancer drugs is crucial, constituting a primary objective for medicinal chemists. A series of novel pyrimidine derivatives were synthesized through the refluxing of (1E, 3E)-4-phenyl-1-(2-phenylhydrazinylidine) but-3-en-2-one with urea using ethanolic acetic acid as a solvent. The chalcone intermediates were prepared by stirring a mixture of aromatic aldehyde and substituted (1E)-1-(2-phenylhydrazinylidine) propan-2-one in the presence of 20% NaOH. The synthesized compounds were characterized using IR, ^{1}H NMR, and Mass spectroscopic studies. Cytotoxicity assays were conducted on lung cancer cell lines utilizing MTT and SRB methods, revealing varying degrees of cytotoxic activity among the compounds. Furthermore, the potential antioxidant properties of the synthesized compounds were evaluated by DPPH, nitric oxide scavenging, and superoxide radical scavenging assays. Several compounds exhibited exceptional antioxidant and cytotoxic activity compared to ascorbic acid and doxorubicin, standard drugs used for comparison. As a result, these novel pyrimidine derivatives hold promise for further development as both anticancer and antioxidant agents.

Keywords: Cancer, Pyrimidine, Chalcones, Cytotoxic, Antioxidant.

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INTRODUCTION

Chemotherapy and cytotoxic medicines remain the main focus of medicinal chemistry research. Cancer associated with cardiovascular and infectious disorders has the highest fatality rates among emerging diseases.[1] Due to their beneficial biological and pharmacological features, most heterocyclic compounds are gaining greater relevance in recent years.[2] The current work has taken novel substituted pyrimidines derived from specific chalcones. The best paths for drug discovery may involve developing novel molecules based on favored scaffolds. Pyrimidines, as such are not found in nature; only substituted pyrimidines and molecules having this ring are distributed in nature. They are the most significant 6-membered heterocyclic molecules having two nitrogen atoms. They are well recognized for various biological activities like anti-tumor[3], analgesic[4], anti-inflammatory[5], antifungal[6], antibacterial[7], antiplatelet[8], cytotoxic[9], antioxidant[10,] and antitubercular activities.[11] Pyrimidine possessing a hydroxyl group holds a special place in medicinal chemistry and is crucial to many biological processes and synthetic chemistry. Chalcones are important biosynthetic intermediates used to create a variety of flavonoids and pharmacologically important heterocyclic moieties such as pyrimidine, pyrazolines, isoxazolines, and benzodiazepines. We intended to synthesize various pyrimidine derivatives obtained from particular novel chalcones and to investigate their biological and pharmacological properties as prompted by the literature survey.

EXPERIMENTAL

Materials and Methods

Analytical-grade chemicals were utilized throughout. By recrystallizing the materials with the appropriate solvents, the products were purified. The digital melting point apparatus was used to determine the

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uncorrected melting points. The purity of both the intermediate and final product was evaluated using thin-layer chromatography (TLC) with silica gel G plates. The spots were exposed to iodine vapors to be seen under UV light. To run TLC, acetone and ethyl acetate (1:6) were employed as the solvent. The characterization of the synthesized compounds was done by IR, ^{1}H-NMR, and mass spectroscopy. Infrared spectra were acquired using an Alpha Bruker IR spectrometer with KBr discs, and the units were expressed in cm^{-1}. Proton nuclear magnetic resonance measurements were conducted using a Bruker Avance-2 operating at 300 MHz, DMSO-d_6 as the solvent, and tetramethylsilane (TMS) as an internal reference. Compared to TMS, chemical shift values were reported in parts per million (ppm). Mass spectra were generated using the SYNAPT-G2 LC-MS spectrometer. The synthesis pathway for the compounds (PYMA1-PYMA8) involved the following steps.

Synthesis of (1E, 3E)-4-phenyl-1(2-phenylhydrazinylidine)but-3-en-2-one (CHH_1-CHH_8)

In the presence of 20% NaOH, 0.01 mol of substituted (1E)-1-(2-phenylhydrazinylidine) propane-2-one and 0.01 mol of aromatic aldehyde were stirred in ethanol for 10 hours. The mixture was filtered, recrystallized using ethanol, and then poured over crushed ice that had been acidified with 5% HCl as given in Fig.-1.

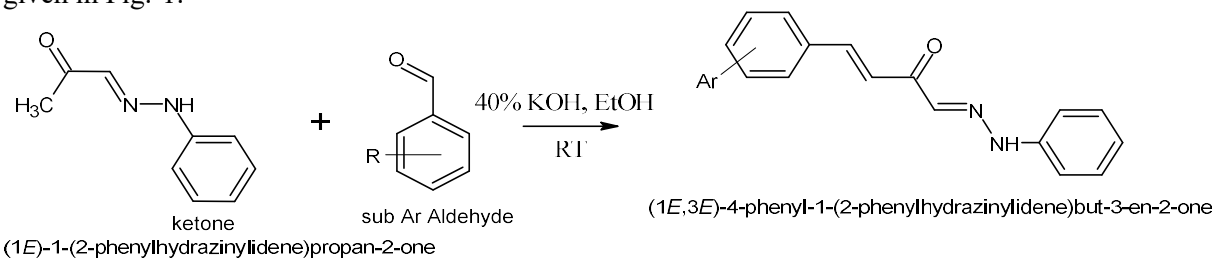

Fig.-1: Synthesis of Chalcone Derivatives

Synthesis of substituted (E)-4-phenyl-6-(2-phenylhydrazono) methyl) pyrimidin-2-ol. (PYMA1-PYMA8)

Substituted (1E, 3E)-4-phenyl-1-(2-phenylhydrazinylidine) but-3-en-2-one (0.01 mol) was made to react with urea (0.01mol) in ethanolic acetic acid and refluxed with 20% NaOH. The resulting product was filtered and then recrystallized from ethanol as given in Fig.-2.

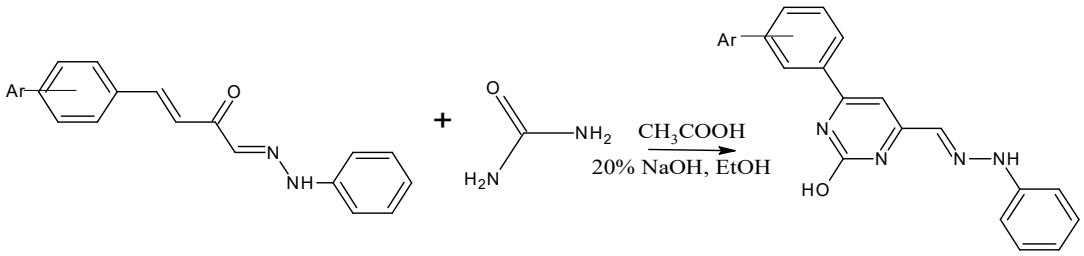

Fig.-2: Synthesis of Pyrimidine Derivatives

Cytotoxic Activity

MTT[12] and SRB[13] methods were utilized to evaluate the compounds' cytotoxicity in an in-vitro setting. The MTT assay relies on living cells converting MTT into formazan crystals to measure mitochondrial activity. The lung cancer (NCIH-460 and NCIH-522) cell lines were used for the study. Various concentrations (ranging from 5 to 100 g/mL) of selected synthetic compounds were administered to cells, which were subsequently cultured at 37°C for 24 hours in the presence of 5% CO_2. Following a 24-hour incubation period and confirmation of the solubilization of purple formazan crystals, 10 µL of MTT labeling reagent was added. Subsequently, the absorbance at 550 and 600nm was measured using a microplate (ELISA) reader. SRB assay relies on the capacity of the sulforhodamine B protein dye to attach to trichloroacetic acid-treated cells, specifically to the basic amino acid residues dependent on the pH. The following procedure was utilized for cell proliferation cytotoxicity analysis: Each well of a 96-well microtiter plate was filled with a volume of 100 µL of cell suspension. The plate was then placed in a 37°C incubator with 5% CO2. Various concentrations (ranging from 5 to 100 µg/mL) of the desired synthesized compounds were added to the wells. Subsequently, the sulforhodamine B (SRB) assay was

performed following the instructions provided by the manufacturer using the EZ count TM sulforhodamine B cell assay kit. Absorbance was measured at 565nm using an ELISA microplate reader. GI_{50} values were calculated using Microsoft Excel. In both cases, Doxorubicin was used as a standard drug.

$$\% \text{ Cell viability} = \frac{Mean\ absorbance\ of\ test}{Mean\ absorbance\ of\ control} X\ 100$$

Antioxidant Activity

The in-vitro antioxidant potential of all the compounds was evaluated using DPPH, nitric oxide scavenging, and superoxide radical scavenging assays.[14]

DPPH Radical Scavenging Activity

This experiment used a spectrophotometric assay using DPPH as the reagent. A solution was prepared by combining 3 mL of 0.1 mM DPPH with 1 mL of synthesized compounds at 10 to 50 µg/mL concentrations. The mixture was then placed in a dark environment and incubated at room temperature for a duration of 30 minutes. Following incubation, absorbance at 517 nm was measured against a blank, using ascorbic acid as the reference substance.[15]

Nitric Oxide Free Radical Scavenging Method

Using sodium nitroprusside, nitric oxide radicals (NO) were generated. A mixture of 1.5 mL of phosphate buffer saline (0.2M, pH 7.4) and 1 mL of sodium nitroprusside (10 mM) was prepared to which different quantities (ranging from 10 to 50 µg/mL) of the test compounds were added. The obtained mixture was subjected to an incubation period of 150 minutes at 25°C. Subsequently, the reaction mixture (1 mL) was mixed with Griess reagent (1 mL), and the combined mixture was subjected to 546 nm to measure the absorbance. Using the formula below, the percentage inhibition was calculated, with ascorbic acid as the standard drug.

$$\% \text{ Inhibition} = \frac{absorbance\ of\ control - absor\quad of\ sample}{absorbance\ of\ control} x\ 100$$

Superoxide Radical Scavenging Assay

A combination of phenazine methosulfate (PMS) and NADH was chosen to generate superoxide radicals. The presence of superoxide radicals leads to the conversion of NBT to formazan, which can be quantitatively assessed using a UV spectrometric method at 560 nm. The ability of the tested compounds to inhibit formazan formation was determined by measuring the decrease in absorbance. The assay was conducted in 96-well microtiter plates. The test samples were prepared by dissolving in dimethylsulfoxide (DMSO), while the standard was prepared by mixing 10 mM of NBT and 50 µL of NADH in phosphate buffer. This mixture was then combined with a 50 µL solution of PMS (10 mM). The mixture was placed in an incubator at a temperature of 25°C for a duration of 5 minutes, after which the absorbance of the compounds was measured at a wavelength of 560 nm. The percentage of inhibition was calculated using a specific formula.[16]

$$\% \text{ Inhibition} = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of control} x100$$

RESULTS AND DISCUSSION

Spectral Data

PYMA-1 (E)-4-phenyl-6-2[(2-phenyl hydrazono) methyl] pyrimidin-2-ol. IR KBr (cm^{-1}): 3307 (N-H str), 3346 (O-H str), 3013 (Ar C-H str), 1558 (C=N str), and 1514 (C=C str) ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.5-8.0 (m, Ar-H, 11H), 6.9 (s, N=C-H, 1H), 9.2 (s, OH, 1H). MS (M^+): m/z 290.1.

PYMA-2 (E)-4–(4-bromophenyl)-6-{(2-phenylhydrazono) methyl} pyrimidin-2-ol. ^{1}H-NMR (300 MHz, DMSO-d_6): 7.3 (s, N=C-H, 1H), 8.5 (s, NH, 1H), and 8.05 (s, OH, 1H) IR KBr (cm^{-1}) 3240 (N-H str), 3069 (Ar C-H str), 1514 (C=N str), 1558 (C=C str), 961 (C-Br str), and 3341 (O-H str). MS (M^+): m/z 369.7.

PYMA-3 (E)-4-((2-phenylhydrazono)methyl)-6-(1H-pyrrol-2-yl)pyrimidin-2-ol. IR KBr (cm^{-1}), N-H str 3228, Ar C-H str 3058, C=N str 1510, C=C str 1552, O-H str 3317. 7.3 (s, N=CH, 1H), 8.3 (s, NH, 1H), and 9.0 (s, OH, 1H) are the values of ^{1}H-NMR (300 MHz, DMSO-d_6). MS (M^+): m/z 279.

PYMA-4 (E)-4-(4-hydroxyphenyl)-6-((2-phenylhydrazono)methyl)pyrimidin-2-ol. IR KBr (cm^{-1}): 3223 for N-H str, 3056 for Ar C-H str, 1506 for C=N str, 1546 for C=C str, and 3309 for O-H str. 7.6–8.1 (m, Ar–H, 10H), 7.5 (s, N=CH, 1H), 8.9 (s, NH, 1H), and 9.6 (s, OH, 1H) are the ^{1}H–NMR results obtained at 300 MHz using DMSO–d_6. MS (M^+): m/z 306.

PYMA-5 (E)-4-(furan-2-yl)-6-((2-phenyl hydrazono)methyl)pyrimidin-2-ol. IR KBr (cm^{-1}) values include 3378 (N-H str), 2972 (Ar C-H str), 1556 (C=N str), 1510 (C=C.str), and 3315 (O-H str). 7.3-8.1 (m, Ar-H, 9H), 7.2 (s, N=C-H, 1H), 8.1 (s, NH, 1H), and 9.3 (s, OH, 1H) are the ^{1}H-NMR (300 MHz, DMSO-d_6) results. MS (M^+): m/z 282.

PYMA-6 (E)-4-(4-dimethylamino phenyl)-6-((2-phenyl hydrazono) methyl) pyrimidin-2-ol. 3228 (N-H-str), 3048 (Ar C-H-str), 1510 (C=N str), 1539 (C=C-str), and 3318 (O-H-str) are the IR-KBr (cm^{-1}) values.^{1}H-NMR-(300MHz,-DMSO-d_6):7.3-6.8 (s, N=C-H, 1H), 8.8 (s, NH, 1H), 9.7 (s, OH, 1H), and 2.6 (s, 2XCH3, 6H). MS (M^+): m/z 380.

PYMA-7 (E)-4-(2-bromophenyl)-6-((2-phenyl hydrazono)phenyl)pyrimidin-2-ol. IR KBr (cm^{-1}): 3327 (O-H str), 1510 (C=N str), 1552 (C=C str), 957 (C-Br str), 3235 (N-H str), and 3052 (Ar C-H str).7.3-8.0 (m, Ar-H, 10H), 7.2 (s, N=CH, 1H), 8.4 (s, NH, 1H), and 9.2 (s, OH, 1H) are the ^{1}H-NMR (300MHz, DMSO-d_6) results. MS (M^+): m/z 369.

PYMA-8 (E)-4-(4-hydroxy-3-methoxy phenyl)-6[(2-phenyl hydrazono) methyl) pyrimidin-2-ol. IR KBr (cm^{-1}): 3319 (O-H str), 1508 (C=N str), 1546 (C=C str), 3251 (N-H str), and 3045 (Ar C-H str). ^{1}H-NMR (300MHz, DMSO-d_6) δ:7.1-8.1 (m, Ar-H, 10H), 7.1 (s, N=C-H, 1H), 8.3 (s, NH, 1H), 9.3 (s, OH, 1H) 3.40 (s, OCH_3, 3H).

Chemistry

Various substituted (1E, 3E)-4-phenyl-1-(2-phenylhydrazinylidene) bute-3-en-2-one were prepared by stirring a mixture of aromatic aldehyde and substituted (1E)-1-(2-phenylhydrazinylidene) propan-2-one in presence of 20% NaOH as given in Table-1. Prepared chalcones were purified by TLC, characterized by melting point, and infrared spectra showed a distinctive absorption peak at 1675 cm^{-1} of the CH=CH group. The title compounds substituted (E)-4-phenyl-6-[(2-phenylhydrazine)methyl] pyrimidine-2-ol (PYMA1-PYMA8) derivatives were prepared by as referred in literature[15] by cyclization of chalcones with urea in ethanolic acetic acid and refluxed in 20% NaOH as given in Table-2. The IR spectra of substituted pyrimidine (PYMA1-PYMA8) show IR absorption at 3410-3460 cm^{-1} because the OH group is absent in chalcones. The singlet at δ 9.1-9.3 is due to the OH group observed in ^{1}H-NMR spectra of the substituted chalcones. Furthermore, the formation of pyrimidine derivatives was unequivocally verified through mass spectral data, which align perfectly with the corresponding molecular formula.

Table-1: Physical Data of Chalcone Derivatives (CHH1-CHH8)

S. No.	R-CHO	Mol Formula	Mol. wt	Yield(%)	MP(˚C)
CHH-1	H	$C_{16}H_{14}N_2O$	250.29	72	126-128
CHH-2	4-Br	$C_{16}H_{13}BrN_2O$	329.18	68	212-214
CHH-3	Pyrrole	$C_{14}H_{13}N_3O$	239.27	64	136-138
CHH-4	4-OH	$C_{16}H_{14}N_2O_2$	266.29	56	165-167
CHH-5	Furan	$C_{14}H_{12}N_2O_2$	240.25	71	130-132
CHH-6	4-N-$(CH_3)_2$	$C_{18}H_{19}N_3O$	293.35	55	165-167
CHH-7	2-Br	$C_{16}H_{13}BrN_2O$	329.18	68	212-214
CHH-8	3-OCH_3,4-OH	$C_{17}H_{16}N_2O_3$	296.31	67	147-149

Table-2: Physical Data of Pyrimidine Derivatives (PYMA1-PYMA8)

S. No.	R_1	Mol Formula	Mol. wt	Yield(%)	MP(˚C)
PYMA-1	H	$C_{17}H_{14}N_4O$	290.31	69	174-177
PYMA-2	4-Br	$C_{17}H_{13}BrN_4O$	369.20	54	164-167

PYMA-3	Pyrrole	$C_{15}H_{13}N_5O$	279.29	36	150-153
PYMA-4	4-OH	$C_{17}H_{14}N_4O_2$	290.31	30	167-170
PYMA-5	Furan	$C_{15}H_{12}N_4O_2$	280.28	69	212-214
PYMA-6	4-N-$(CH_3)_2$	$C_{19}H_{19}N_5O$	333.38	56	227-229
PYMA-7	2-Br	$C_{17}H_{13}BrN_4O$	369.20	70	210-212
PYMA-8	3-OCH_3,4-OH	$C_{18}H_{16}N_4O_3$	304.34	54	228-230

Cytotoxic Studies

The test compounds were subjected to *in-vitro* cytotoxicity using MTT and SRB assay with two lung cancer cell lines, NCIH-460 and NCIH-522. Purple formazan crystals formed were confirmed by measuring the absorbance at 550 and 600nm using a microplate (ELISA) reader. Table-3 demonstrates that compounds PYMA2, PYMA6, PYMA7, and PYMA8, which incorporate a bromo phenyl group, exhibited significant anticancer activity. The remarkable cytotoxic impact can be ascribed to the existence of electron-withdrawing groups within these compounds.

Table-3: GI_{50} Values of Pyrimidine Derivatives for their Anti-Cancer Activity

Compound	NCIH 460		NCIH 522	
	MTT	SRB	MTT	SRB
PYMA-1	32.8±1.45	71.11±4.32	175.42±9.36	71.11±1.6
PYMA-2	109.3±2.5	131.27±7.71	74.61±4.69	26.27±8.68
PYMA-3	123.82±5.84	65.58±5.18	52.44±6.99	65.58±7.27
PYMA-4	73.19±5.25	65.45±4.95	67.33±7.12	65.45±7.68
PYMA-5	57.38±7.42	59.32±3.1	96.46±7.59	69.21±3.89
PYMA-6	11.16±8.13	112.23±9.92	42.31±2.43	169.48±2.44
PYMA-7	132.12±9.5	33.09±9.27	86.13±9.4	43.16±4.79
PYMA-8	269.86±5.69	52.29±4.11	19.97±3.26	130.19±3.54
Std (Doxorubicin)	14.69±9.86	21.14±9.53	16.21±9.62	19.52±9.09

Antioxidant Activity Studies

To evaluate the in-vitro antioxidant activity of the compounds, DPPH scavenging, nitric oxide, and superoxide radical scavenging methods were employed. A reference standard ascorbic acid was utilized. Notably, compounds PYMA1 and PYMA8 exhibited remarkable antioxidant properties, surpassing the effectiveness of the standard drug ascorbic acid, as depicted in Table-4.

Table-4: Antioxidant Activity of Pyrimidine Derivatives (IC_{50} Value)

Compound	DPPH	Nitric Oxide	Superoxide
PYMA-1	36.25±5.89	4.46±3.75	242.69±4.37
PYMA-2	29.4±2.85	66.39±3.85	63.88±2.28
PYMA-3	49.37±2.28	150.8±2.76	231.69±5.76
PYMA-4	225.28±5.62	45.28±7.73	239.62±6.03
PYMA-5	165.62±4.6	70.84±3.39	161.43±7.29
PYMA-6	129.17±4.46	74.46±6.19	118.83±3.75
PYMA-7	16.23±5.44	36.24±3.89	14.78±3.96
PYMA-8	59.58±3.85	157.69±6.16	92.06±3.63
Std (Ascorbic acid)	7.16±1.21	16.63±2.23	3.72±0.87

CONCLUSION

The present study presents the successful synthesis of various substituted novel pyrimidine derivatives with an average yield of 50-70%. The cytotoxic activity of compounds (PYMA1-PYMA8) was evaluated using MTT and SRB assay methods at different concentrations (5-100 µg/mL), and compounds PYMA2, PYMA6, PYMA7, and PYMA8 showed significant anticancer activity compared to ascorbic acid as standard drug. Based on the above result, it can be concluded that compounds PYMA1, PYMA6, PYMA7, and PYMA8, with further modification can be a promising lead molecule for *in-vivo* anticancer studies.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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